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# Role of TC-PTP-mediated regulation of STAT1 in skin carcinogenesis

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**Title:** Role of TC-PTP-mediated regulation of STAT1 in skin carcinogenesis

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## Abstract:

STAT1 has been shown to have tumor suppressive or protumorigenic roles in tissues in a context-dependent manner. In this study, we explored the role of TC-PTP in the regulation of STAT1 during skin tumor promotion. We found that the levels of the phosphorylated serine form of STAT1 were increased in TC-PTP overexpressing keratinocytes in response to TPA treatment. Additionally, TC-PTP overexpression decreased TPA-induced proliferation through regulation of STAT1 signaling. These results suggest that positive regulation of STAT1 signaling via TC-PTP can have a protective role towards keratinocytes during tumor promotion. These findings suggest that targeting TC-PTP-mediated regulation of STAT1 signaling could have therapeutic implications for treating or preventing skin cancer.

## Introduction

Signal transducers and activators of transcription (STATs) are important transcription factors that have roles in the regulation of gene expression for particular genes involved in cell proliferation, differentiation, apoptosis, and homeostasis among other functions [1, 2]. The STAT family is composed of seven family members to date including STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6. STAT1, the first identified STAT family member, has two isoforms, STAT1-α and STAT1-β which are generated by alternative splicing. STAT1-β lacks a serine phosphorylation site and much of the transactivation domain, whereas STAT1-α is transcriptionally active and encodes a 750 amino acid protein [3].

STAT1 has been identified as an essential component of IFN-related signaling [4, 5]. Type I-III interferons activate STAT1 [6]. STAT1 is activated when it's phosphorylated on conserved tyrosine (tyr701) and serine (ser727) residues within the transactivation domain. This activation can trigger dimerization, resulting in translocation to the nucleus and regulation of gene expression [7-9]. Through its activation and modulation of target gene expression, STAT1 plays as an important role in a range of biological processes including roles in innate and adaptive immunity leading to protection against viral or bacterial infections as well as playing a role in modulating tumor development and progression [6, 10-12].

The role of STAT1 in immunity and cancer development are of great interest; however, opposing findings regarding the role of STAT1 in cancer have been reported. Classically, activated STAT1 (pSTAT1) has been described as antitumorigenic due to its influence on tumor immune surveillance and regulation of apoptotic and cell cycle factors [4, 11-15]. The antitumorigenic action of STAT1 is illustrated by studies finding that STAT1 expression is often lost in various human cancers including breast cancer, esophageal cancer, colon cancer, and

pancreatic cancer, among other types of cancers[16-20]. However, protumorigenic functions have also been recently attributed to STAT1. A protumorigenic role of STAT1 signaling has been illustrated in breast cancers, esophageal cancer, colon cancer, melanoma, and leukemia [18, 21-27]. These conflicting findings suggest that STAT1 may be capable of mediating tumor suppressor or protumorigenic behavior depending on the cell type, stimuli, and context that are present[4, 10].

Our interest lies in the role of STAT1 in skin carcinogenesis, especially considering its interplay with phosphotyrosine-based signaling. This lab has a particular interest in T-cell protein tyrosine phosphatase (TC-PTP), an intracellular, non-receptor PTP that is involved in several processes such as cell proliferation, differentiation, and apoptosis via its regulation of its target substrates which include JAK1, JAK3, STAT1, and STAT3 [28-30]. This lab has done extensive work regarding the role of TC-PTP in skin carcinogenesis and has found evidence that TC-PTP functions as a tumor suppressor in vivo and in vitro in response to both UV and chemically-induced skin carcinogenesis [31, 32]. This tumor suppressive action is attributable to its ability to suppress STAT3 and Flk-1/JNK signaling [31, 32]. Interestingly, another lab recently identified a protumorigenic role of the STAT1 signaling pathway in keratinocytes following solar UV exposure[10]. Due to the lack of clarity regarding the role of STAT1 in skin carcinogenesis, we chose to explore the role of STAT1 in skin carcinogenesis in the setting of TC-PTP overexpression.

## Materials & Methods

### Keratinocyte cell culture

Immortalized keratinocytes that overexpress TC-PTP were generated as previously described by this lab [33]. Immortalized keratinocytes were plated and cultured at 37°C and 5% $CO_2$ in low $Ca^{2+}$ KGM-2 medium containing 1% penicillin/streptomycin and 1% fetal bovine serum until 60-70% confluent. When cells reached 60-70% confluency they were treated with TPA.

### Western blot analysis

Cells were washed three times with ice-cold phosphate buffered saline (PBS) before lysis. Cells were resuspended with lysis buffer containing 40 mM Tris, pH: 7.4, 120 mM NaCl, 10 mM EDTA, 0.1% (v/v) NP-40, protease inhibitor cocktail, phosphatase inhibitor cocktail 1 and 2 (P8340, P2850 and P5726; Sigma-Aldrich, St. Louis, MO). Then cells were lysed with three freeze and thaw cycles. Lysates were then clarified by centrifugation at 12,000 rpm at 4° C for 30 minutes and protein content analysis was performed using the DC protein assay (Bio-Rad Laboratories, Inc., Hercules, CA). Equal amounts of protein were separated by NuPAGE® Novex® 4-12% Bid-Tris Gel (Invitrogen, Carlsbad, CA), and were transferred to PVDF membrane (GE Healthcare Life Sciences, Pittsburgh, PA). The membrane was incubated overnight at 4°C with primary antibody, followed by incubation with a horseradish peroxidase-conjugated secondary antibody. Chemiluminescent detection reagents (GE Healthcare Bio-Sciences Co., Piscataway, NJ) were used to detect immunoreactive protein.

## Results

*TC-PTP overexpression is associated with decreased TPA-induced proliferation via STAT1 regulation*

The regulatory role of TC-PTP was explored during treatment with TPA, a tumor promoter. To observe the initial response to TPA-treatment, primary keratinocytes from WT and TC-PTP overexpressing mice were treated with TPA for 1 hour and then were cultured for 4 hours. A significant increase in pY-STAT1 expression levels was observed in control keratinocytes compared to TC-PTP overexpressing keratinocytes following TPA treatment, whereas a significant increase in pS-STAT1 levels was seen in TC-PTP overexpressing keratinocytes after treatment with TPA (Fig. 1a).

To investigate the long-term response to TPA treatment, primary keratinocytes from WT and TC-PTP overexpressing mice were treated with TPA for 1 hour and then cultured for 72 hours. Western blot analysis showed no visible expression of pY-STAT1 in either keratinocyte type; however, there was increased pS-STAT1 24 hours after treatment with TPA in both keratinocyte types. The pS-STAT1 expression levels were found to be higher in TC-PTP overexpressing keratinocytes compared to the WT keratinocytes, while total STAT1 levels were lower in the TC-PTP overexpressing keratinocytes compared to control keratinocytes (Fig. 1b).

Inhibition of STAT1 prior to TPA treatment significantly increased the cell viability in TC-PTP overexpressing keratinocytes (Fig. 1c). These findings together suggest that TC-PTP-mediated serine phosphorylation of STAT1 has an inhibitory effect on TPA-induced cell proliferation. This suggestion is further bolstered by the finding that TC-PTP overexpressing cells treated with a STAT1 inhibitor prior to TPA treatment showed cell cycle progression with increased cells in S and G2/M phases on flow cytometry. These findings are supportive of the suggestion that TC-PTP plays a protective role towards keratinocytes during tumor promotion with TPA through positive regulation of STAT1 signaling leading to inhibition of TPA-induced cell proliferation.

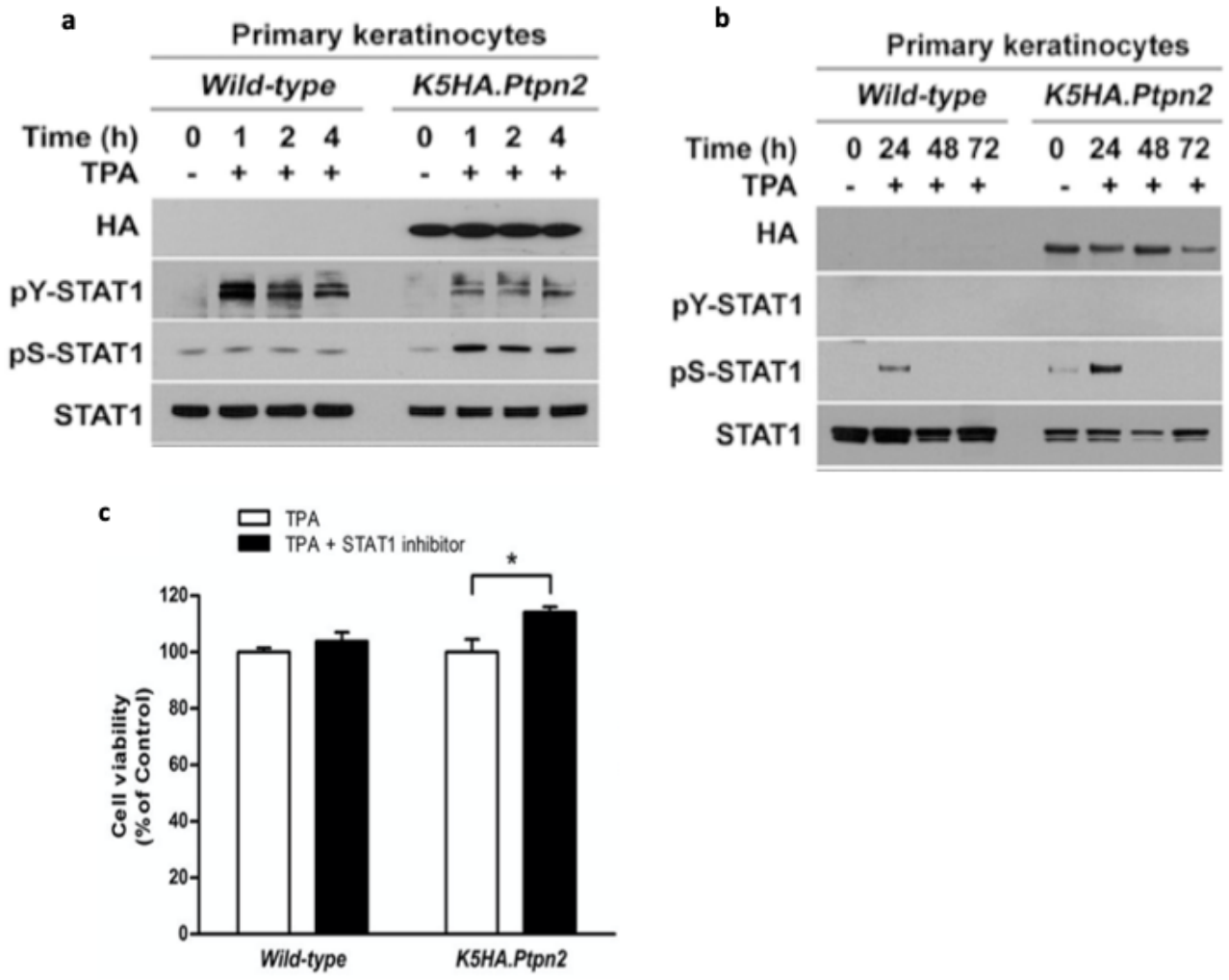


**Figure 1. Reduction of TPA-induced cell proliferation through STAT1 regulation in TC-PTP overexpressing keratinocytes**

**a)** Western blot analysis to evaluate initial response to TPA treatment. Analysis of STAT1 in primary keratinocytes from wild-type and epidermal-specific TC-PTP overexpressing mice following TPA treatment.

**b)** Western blot analysis to evaluate long-term response to TPA treatment. Analysis of STAT1 in primary keratinocytes from wild-type and epidermal-specific TC-PTP overexpressing mice following TPA treatment.

**c)** Analysis of cell viability after TPA treatment in wild-type and TC-PTP overexpressing keratinocytes. Keratinocytes were treated with a STAT1 inhibitor prior to TPA treatment and cell viability was measured using WS-assay after 24 hours of TPA treatment. *$p<0.05$ by ANOVA

## Discussion

The role of STAT1 and its implications for cancer have been studied for over a decade, but the complexities of its role as a tumor suppressor or tumor promoter in a context-dependent manner still require elucidation [35]. Previous studies have shown STAT1 activation in keratinocytes plays a protumorigenic role in skin as evidenced by solar UV radiation induced epidermal proliferation and hyperplasia in addition to dermal inflammation [10]. Meanwhile

our current research investigating the interplay between TC-PTP and STAT1 in skin carcinogenesis, showed evidence that serine phosphorylation of STAT1 promoted apoptosis in keratinocytes, and the pS-STAT1 levels increased in TC-PTP overexpressing keratinocytes in response to treatment with TPA, a tumor promoter. These findings suggest that in the context of chemical skin carcinogenesis using TPA, the positive regulation of STAT1 serine phosphorylation by TC-PTP provides protection against tumor promotion.

In the future, the modulation of the expression or activation of TC-PTP or its target STAT1 could be used to prevent or treat skin cancer. However, more research is needed regarding the role of STAT1 in carcinogenesis to identify how STAT1 regulation could be used therapeutically. Additional research into the implications of serine phosphorylation versus tyrosine phosphorylation of STAT1 could also provide clarification for how STAT1 can be used as a target for immune or cancer modulation. In order to better understand the role of STAT1 in skin carcinogenesis, further elucidation is needed to clarify its signaling pathway and downstream effects as well as how it interacts with and impacts other signaling pathways.

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